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Electroretinography in Dogs and Cats. Part I. Retinal Morphology and Physiology

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Abstract
Electroretinography is an important objective procedure that is used to assess the outer retina and follow the progression of and recovery from retinal disorders. This procedure is more sensitive than other diagnostic techniques, such as ophthalmoscopy, for determining subtle or early alterations in the outer retina. Electroretinography cannot, however, assess vision because an electroretinograpn (ERG) may be normal in dogs and cats with cortical blindness or early stages of glaucoma. If retinal dysfunction is known or suspected, an ERG may be necessary. This two-part presentation provides general practitioners with information about this relatively noninvasive electrodiagnostic procedure in order to assist them in assessing the need for referral to a veterinary ophthalmologist or neurologist. Part I reviews the morphologic and physiologic characteristics of the retina; Part II will examine electroretinographic technique, interpretations, and indications.

Disciplines
Eye Diseases | Medicine and Health Sciences | Ophthalmology | Veterinary Medicine

Comments
Dr. Komáromy was affiliated with the University of Pennsylvania from 2003-2012.

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CE Article #6 (1.5 contact hours)
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Electroretinography in Dogs and Cats. Part I. Retinal Morphology and Physiology

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FOCAL POINT

- Electroretinography is indicated when retinal dysfunction must be confirmed or ruled out.

KEY FACTS

- Electroretinography is more sensitive than other diagnostic techniques in determining subtle or early alterations in the outer retina, p. 343.
- Although it cannot assess vision, an electroretinogram can assist practitioners in evaluating the cause of blindness, p. 343.
- Rods outnumber cones in canine and feline retinas, p. 344.
- The electroretinogram is a mass response that measures the summation of the changes of membrane potentials in the entire retina, p. 348.
- The pathways of rods and cones must be evaluated separately, p. 348.

Electroretinography is an important objective procedure that is used to assess the outer retina and follow the progression of and recovery from retinal disorders. This procedure is more sensitive than other diagnostic techniques, such as ophthalmoscopy, for determining subtle or early alterations in the outer retina. Electroretinography cannot, however, assess vision because an electroretinogram (ERG) may be normal in dogs and cats with cortical blindness or early stages of glaucoma. If retinal dysfunction is known or suspected, an ERG may be necessary. This two-part presentation provides general practitioners with information about this relatively noninvasive electrodiagnostic procedure in order to assist them in assessing the need for referral to a veterinary ophthalmologist or neurologist. Part I reviews the morphologic and physiologic characteristics of the retina; Part II will examine electroretinographic technique, interpretations, and indications.

MORPHOLOGIC CHARACTERISTICS OF THE RETINA

The retina has 10 layers that can be identified on histologic examination (Figure 1).[1] The following brief discussion reviews these layers from the outside (i.e., closer to sclera) inward.

The retinal pigment epithelium (RPE) consists of a layer of flat polygonal cells located between the choroid and the photoreceptor layer (Figure 1). Because the RPE adheres more closely to the choroid than to the remaining retinal tissue, there may be a space between the RPE and the photoreceptor layer (the remnant of the optic vesicle). Most retinal detachments or separations occur in this so-called subretinal space, which actually is an intraretinal space. The RPE cells have several important functions, including the transport of nutrients from the choroid to the outer layers of the retina, phagocytosis of outer

segments of photoreceptors as they are shed, and maintenance of retinal attachment by creating a gradient for the fluid that flows from the subretinal space. The villous cytoplasmic processes of the RPE cells surround the photoreceptors, insulate them from bright light, and increase their individual sensitivity to light. The RPE cells usually are densely pigmented but are devoid of pigment in the dorsal region of the RPE overlying the choroidal tapetum. In dogs and cats, the tapetum lucidum is a cellular structure that is located within the choroid (external to the retina) and that reflects light back through the retina. The reflected light results in greater stimulation of the photoreceptors, thereby enhancing night vision.

The remaining nine retinal layers represent the neurosensory portion of the retina. The photoreceptor layer contains the inner and outer segments of rod and cone photoreceptors (Figure 1). These segments are closely packed together parallel to the incoming light in a radial fashion. Rods are narrower and longer than cones, except in the central retina. The outer segments of rods and cones contain light-absorbing pigmented molecules (rhodopsin in rods) located in disk-shaped, double-sided membranes (Figure 2).[2] Rods outnumber cones in dogs and cats.[3,4] The number of rods is greatest toward the periphery of the retina; and the number of cones is greatest in the area centralis of the central retina, which provides better visual acuity.[3,4] In cats, the ratio of rods to cones is as low as 11:1 in the area centralis; however, the ratio reaches plateaus of about 65:1 in the periphery and 100:1 near the ora serrata.[4] In general, the density of neurons in all layers decreases toward the periphery.[5] Disease of the rods and cones is prominent in dogs and cats with primary inherited degenerations of the retina (e.g., progressive retinal atrophy).[6]

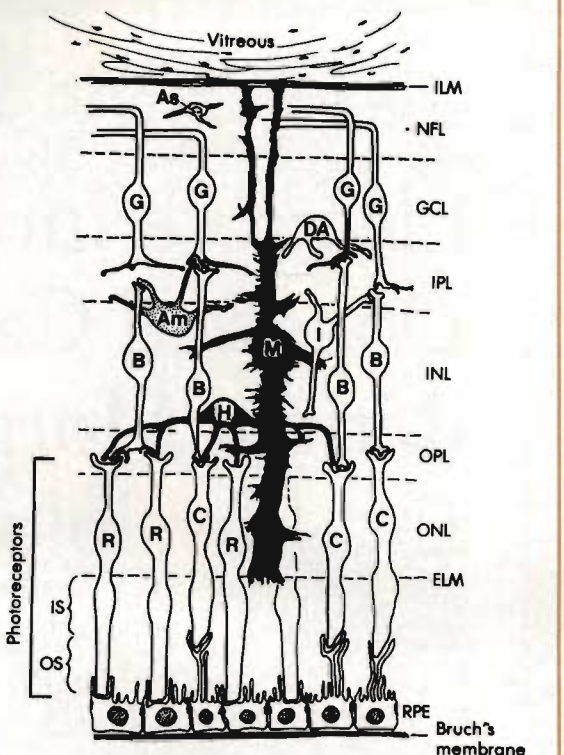

Figure 1—Schematic diagram of the cell types and histologic layers in the retina. Bruch's membrane (basement membrane of the retinal pigment epithelium [RPE]) and the edge of the vitreous are also shown. The basic relationship between rod (*R*) and cone (*C*) photoreceptors as well as bipolar (*B*), horizontal (*H*), amacrine (*Am*), interplexiform (*I*), displaced amacrine (*DA*), and ganglion (*G*) cells is depicted. Note that the Müller cell (*M*) extends almost the entire width of the retina; the apical processes of the Müller cell form the external limiting membrane (*ELM*). Astrocytes (*As*) are found primarily in the nerve fiber layer (*NFL*). *GCL* = ganglion cell layer, *ILM* = internal limiting membrane, *INL* = inner nuclear layer, *IPL* = inner plexiform layer, *IS* = inner segment of photoreceptor cells, *ONL* = outer nuclear layer, *OPL* = outer plexiform layer, *OS* = outer segment of photoreceptor cells. (From Blanks JC: Morphology of the retina, in Ryan SJ (ed): *Retina*, vol 1, ed 2. St. Louis, CV Mosby Co, 1994, p 39. Reprinted with permission.)

The external limiting membrane is composed of tight junctions located between extensions of Müller cells; it separates the inner segments and the nuclei of the photoreceptors (Figures 1 and 2). The outer nuclear layer consists of rows of photoreceptor nuclei. The central retina has the greatest number of these rows, which gradually become thinner in the peripheral retina as the density of rods and cones decreases.[5] The axons of the rods and cones and the dendrites of cells of the inner nuclear layer form the outer plexiform layer (Figure 1). The nuclei of bipolar, amacrine, horizontal, interplexiform, and Müller cells are located in the inner nuclear layer (Figure 1). Müller cells, which are glial cells that provide nutrition and support for the retina, are elongated, branching cells that extend from the internal limiting membrane to beyond the external limiting membrane and fill almost all extracellular space between neurons. Horizontal, bipolar, amacrine, and interplexiform cells are neurons that are connected to each other and that interconnect photoreceptors. The axons of amacrine and bipolar cells synapse with dendrites of ganglion cells in the inner plexiform layer (Figure 1).

The ganglion cell layer, which contains retinal ganglion and neuroglial cells (Figure 1), is the innermost cellular layer of the retina. This single layer of cells is sparse except in the area centralis, where it can be two to three layers thick.[5] Axons of ganglion cells course toward the optic nerve head in the nerve fiber layer (Figure 1).

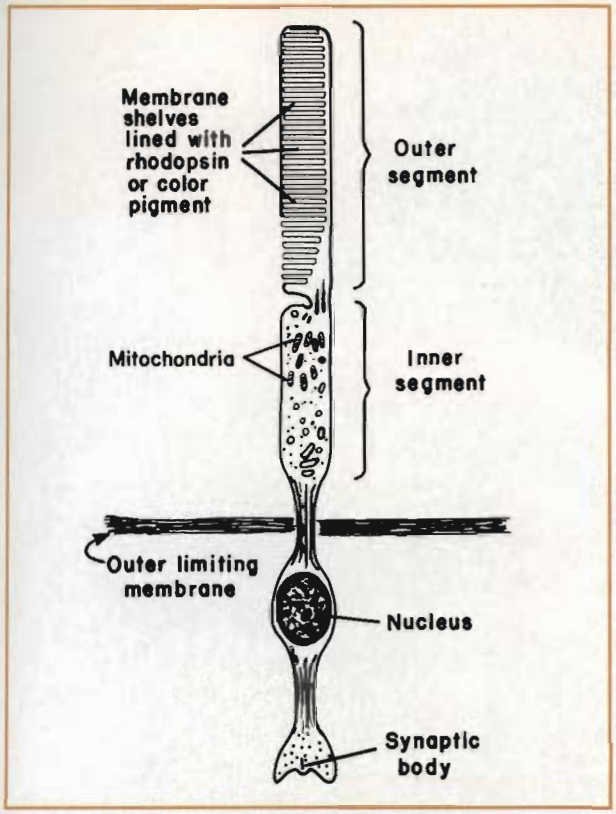

Figure 2—Schematic drawing of the functioning parts of a photoreceptor cell. Note how the orientation differs from that shown in Figure 1. (From Guyton AC: *Textbook of Medical Physiology*, ed 7. Philadelphia, WB Saunders Co, 1986, p 712. Reprinted with permission.)

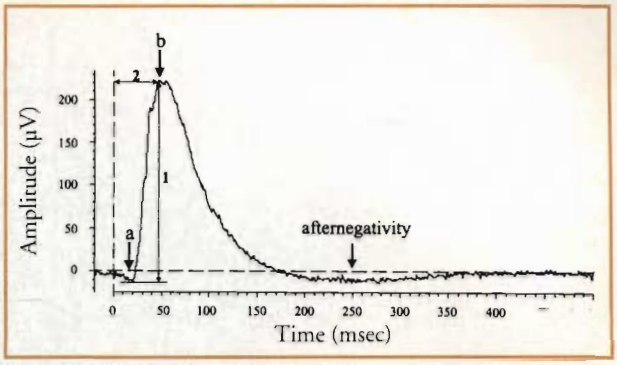

Figure 3—This normal flash electroretinogram of a cat shows the negative a-wave (*a*) and the positive b-wave (*b*) as well as the afternegativity. The positive c-wave is not visible on this recording. The amplitude (*1*) and implicit time (*2*) of the b-wave are indicated. The b-wave amplitude is measured from the peak of the a-wave to the peak of the b-wave. The implicit time is the time from the light flash (0 msec) to the b-wave peak. Oscillatory potentials are responsible for the notching of the b-wave peak.

The basement membrane of Müller cells forms the innermost layer of the retina, which is the internal limiting membrane (Figure 1).

In dogs and cats, the retina has a holangiotic vascular pattern that accommodates direct blood supply to most of the sensory retina. In cats, three pairs of cilioretinal arteries and veins originate around the periphery of the optic disk. In dogs, 15 to 20 cilioretinal arteries and three to four major veins radiate from the optic disk. Additional smaller veins join the larger veins on the optic disk of dogs. The larger vessels arc around the area centralis, thereby leaving what appears on ophthalmoscopic examination to be a vessel-free zone; in reality, however, a capillary network is present. In dogs, the major retinal arteries and veins lie in the nerve fiber layer and ganglion cell layer and usually do not bulge into the vitreous body.[7] Two dense vascular plexuses are formed by the smaller arterioles, capillaries, and venules: an inner vascular plexus located in the ganglion cell layer and an outer vascular plexus located between the inner nuclear layer and the outer plexiform layer.[7] Between these two plexuses lies a third rudimentary vascular plexus in the inner plexiform layer.[7] The bilaminar vascular pattern disappears toward the ora serrata, with only one vascular plexus in the ganglion cell and inner plexiform layers.[7] Near the optic disk (peripapillary area), a supplementary capillary plexus is present in the thickened nerve fiber layer.[7] Blood vessels are completely lacking in the outer layers of the retina (i.e., from the outer nuclear layer up to and including the RPE).[7] Therefore, these layers receive oxygen and nutrients primarily through diffusion from the choroid. If retinal detachment occurs, such diffusion to the photoreceptors subsides and the retinal cells atrophy.

PHYSIOLOGIC CHARACTERISTICS OF THE RETINA

Because rods function more effectively than do cones in low or scotopic illumination, the rods are well suited for night vision. They produce considerably lower visual acuity (resolution) in several shades of gray; however, rods are useful for motion detection. In comparison, cones are useful for vision during daylight or photopic illumination and can rapidly adapt to repetitious stimuli; however, cones are less sensitive to light and therefore do not respond to low levels of illumination. Cones are responsible for color vision. In addition, because of the minimal degree of convergence found in the neural connections with inner retinal layers, cones can provide better resolution than rods can. Because only rods can function at scotopic levels of illumination and cones do not respond to these levels, color vision is minimal in very dim light. In contrast, cones can func-

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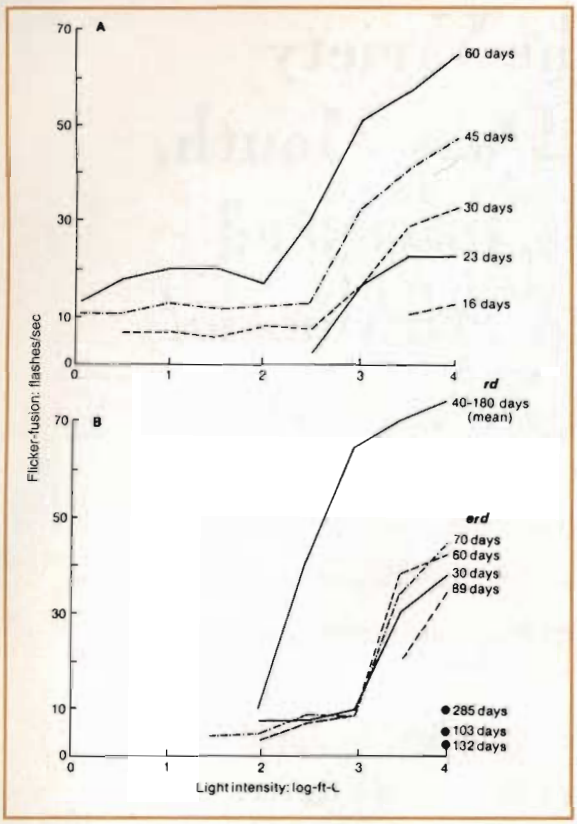

Figure 4—Flicker-fusion frequency (1 flash/sec = 1 Hz) as a function of light intensity of the stimulus (log-ft-L; units of luminance: 1 foot-lambert [ft-L] = 3.426 candelas/m^2 [cd/m^2]) in the retinal development of *(A)* control dogs and *(B)* dogs with early retinal degeneration *(erd)*. The flicker-fusion frequency is defined as the frequency of the flickering light at which flickers are perceived to fuse into a constantly illuminated light. *(A)* In young dogs, flicker responses can be elicited by high-intensity stimuli only and the curve contains what is referred to as the *cone branch*. By 30 days of age, the curve is bipartite, with a rod branch at lower light intensities and an angle or break point between the rod and cone branches. With time, peak fusion frequencies increase at each intensity level and the break point shifts toward the left. *(B)* Dogs with erd have a bipartite curve, but the break point between the rod and cone branches is displaced toward the right on the intensity axis. The rod branch never matures and is lost by 89 days of age. The cone branch degenerates slowly, and responses in older affected animals (103 to 285 days of age) can only be recorded by using maximum light intensities. For comparison, flicker-fusion data for Norwegian elkhounds with rod dysplasia *(rd)* are included. In dogs with this disease, the rod branch cannot be recorded and the cone branch is normal. (From Acland GM, Aguirre GD: Retinal degeneration in the dog: IV. Early retinal degeneration [erd] in Norwegian elkhounds. *Exp Eye Res* 44(4):501, 1987. Reprinted with permission. Comparative data for rod dysplasia obtained from Aguirre GD: Retinal degeneration in the dog: I. Rod dysplasia. *Exp Eye Res* 26(3):233–253, 1978.)

tion and rods become saturated at photopic levels of illumination, thereby making color vision possible.

Cones contain pigments that absorb and respond best to light energy within a narrow segment of the visible light spectrum (wavelength of about 400 to 700 nm). Several studies suggest the presence of two types of cones in the retina of dogs and cats, with maximum absorptive sensitivities in the violet and yellow-green spectra.[8–11]

When the retina is subjected to dark environments, cations (primarily Na^+ and Ca^{2+}) continually leak from the extracellular space into the outer photoreceptor segment through light-sensitive cation channels. At the same time, Na^+ and Ca^{2+} are pumped from the inside of the inner photoreceptor segment to maintain the ionic concentration gradient. The absorption of photons by pigment (e.g., rhodopsin) in disk membranes causes the closure of light-sensitive cation channels in the cell membrane. Such channel closure decreases the amount of cations that are leaking into the outer segment and hyperpolarizes the photoreceptor cell (i.e., more negative membrane potential) because cations continue to be pumped from the inner segment. This transformation of light into electric current is called *phototransduction*.[12] After phototransduction occurs, a series of

TABLE I
Conditions That Favor the Isolation of Responses

Response of Rods	*Response of Cones*
Adaptation to dark	Adaptation to light
Weak stimuli	Intense stimuli
Low flicker rate	High flicker rate

events that involve excitation and inhibition of various neural cells takes place within the retina. The recorded summation of all these changes of electrical membrane potentials in the entire retina as a function of time is the ERG (Figute 3). Because both rod and cone pathways contribute to an ERG, the responses of rods and cones must be considered separately to evaluate retinal dysfunction. The following factors must be considered.[13]

First, rods and cones have different spectral sensitivities. Rods are most sensitive to green (506 nm in dogs and 501 nm in cats), whereas cones are most sensitive to violet (429 to 435 nm in dogs and 450 nm in cats) and yellow-green (555 nm in dogs and cats).[8-11,14] Clinically, longer wavelengths (e.g., red) are used for evaluating the physiologic responses of cones and shorter wavelengths (e.g., blue) for evaluating the responses of rods, even though the overlapping spectral sensitivities of rods and cones make a differentiation with light of different wavelengths almost impossible.

The maximum sensitivity of rods is much greater than that of cones. Because the maximum sensitivity of rods requires adaptation to total dark, the light levels that are used to detect the responses of rods are lower than the levels needed to detect the responses of cones.

The sensitivity of rods decreases when background illumination is added. In contrast, cones can adapt to additional light faster and can continue to maintain the same degree of sensitivity. Adaptation to light depends on the ability of the photoreceptor to reopen light-sensitive cation channels by decreasing Ca^{2+} intracellular concentration in the photoreceptor cells. Such feedback, which is often referred to as Ca^{2+} feedback, is believed to be accelerated in cones and not in rods.[12]

Rods and cones vary in the way they respond to flickering light.[15] The frequency of flickering light at which the flickers are perceived to fuse into a constantly illuminated light is called *flicker-fusion frequency.*[16] If the flicker-fusion frequencies are recorded as a function of light intensity, a flicker fusion response curve is obtained (Figure 4).[17,18] Rods lose the ability to respond to individual flickers of light at a much lower frequency (below 20 Hz) than do cones. The flicker-fusion response curve is normally bipartite; that is, flicker-fusion frequencies of rods occur at lower light intensities and those of cones at higher light intensities. Clinically, flicker-fusion tests further characterize rod and cone function.

The conditions that favor the isolation of rod and cone responses are summarized in Table I.

SUMMARY

Familiarity with the structures of the retina and an understanding the functions of cones and rods are essential when evaluating diagnostic ERGs. Part I of this two-part presentation provides practitioners with the knowledge required to examine the retina; the indications for and technique of performing an ERG as well as its interpretation are discussed in Part II.

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ARTICLE #6 CE TEST

The article you have read qualifies for 1.5 contact hours of Continuing Education Credit from the Auburn University College of Veterinary Medicine. *Choose only the one best answer* to each of the following questions; then mark your answers on the test form inserted in *Compendium.*

1. Retinal detachment leads to degeneration of photoreceptor cells primarily because
   a. the rods and cones are mechanically damaged.
   b. blood flow through the retinal blood vessels of the detached retina decreases.
   c. the rods and cones are more exposed to light.
   d. oxygen and nutrient diffusion from the choroid decreases.

2. Which of the following statements about photoreceptor cells is false?
   a. The outer segments of rods and cones contain light-absorbing molecules.
   b. Progressive retinal atrophy primarily affects photoreceptor cells.
   c. Inner segments can be found in rods but not in cones.
   d. Rods are usually narrower and longer than cones.

3. Which of the following statements about RPE is true?
   a. RPE cells transport nutrients from the choroid to the outer layers of the retina.
   b. The potential space between the RPE and the photoreceptor layer is known as the *subretinal space.*
   c. RPE cells are normally devoid of pigment over the choroidal tapetum.
   d. all of the above

4. Which statement about the flicker-fusion response is incorrect?
   a. Flicker-fusion frequencies change with light intensity.
   b. Rods respond better to flickering light above 20 Hz.
   c. Flicker-fusion frequencies of rods are recorded at low light intensities.
   d. Flicker-fusion tests are clinically useful to characterize the function of rods and cones.

5. Which of the following statements about the retina is incorrect?
   a. Rods outnumber cones in dogs and cats.
   b. The number of rods is greatest toward the periphery of the retina, whereas the number of cones is greatest in the area centralis.
   c. In cats, the ratio of cones to rods is about 11:1 in the area centralis and 65:1 in the periphery of the retina.
   d. The density of all layers decreases toward the periphery.

6. Which statement about the spectral sensitivity of rods and cones in dogs and cats is true?
   a. Rods are most sensitive to green light and cones to either violet or yellow-green light.
   b. The spectral sensitivities of rods and cones overlap.
   c. Rods are most sensitive to blue light and cones to either violet or yellow-green light.
   d. a and b

7. Which of the following methods allows the clinical differentiation of rods and cones?
   a. variation of flicker rates
   b. adaptation to light and dark
   c. variation in light intensity of the stimulus
   d. all of the above

8. Which of the following statements about Müller cells is true?
   a. The nuclei of Müller cells are located in the inner nuclear layer.
   b. Müller cells form the external limiting membrane.

c. Müller cells have nutritive and supportive functions.
d. all of the above

9. Which of the following statements about the canine and feline retina is false?
   a. The retina consists of the RPE and the nine layers that form the neurosensory portion of the retina.
   b. Most of the sensory retina receives a direct blood supply.
   c. The inner nuclear layer contains the nuclei of the retinal ganglion cells.
   d. The axons of the retinal ganglion cells are located in the nerve fiber layer.

10. Which of the following statements about photoreceptor cells is true?
   a. Rods function more effectively in scotopic illumination.
   b. Cones provide better visual acuity.
   c. Rods can rapidly adapt to repeated stimuli.
   d. a and b

Dermatoses *(continued from page 283)*

8. Epitheliotropism of atypical lymphocytes is pathognomonic for which of the following diseases?
   a. cutaneous T-cell lymphoma
   b. vasculitis
   c. SLE
   d. lymphomatoid granulomatosis

9. Cutaneous vasculitis may be associated with
   a. neoplasms.
   b. drug reactions.
   c. infectious diseases.
   d. all of the above

10. Which of the following statements about antinuclear antibody tests is true?
   a. They must be interpreted with caution.
   b. Low titers can be present in old animals.
   c. Low titers may be present in animals with various diseases.
   d. all of the above